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Effect of Maize Silage Inoculated by Bacteria on Performance of Lactating Buffaloes

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Abstract: Two weeks from their parturation, nine lactating buffaloes were used in a 90-d comparative feeding trial to study the effect of uninoculated or inoculated maize silage with Lactic Acid Bacteria (LAB) on their performance. The inoculants were pioneer 1132 which were composed of 100 billion CFU g^{-1} of crop specific *Lactobacillus plantarum* and *Enterococcus faecium*. Animals were similarly grouped into three feeding treatments, according to their weight and milk yield using complete randomized block design. They were introduced to rations containing 60:40 concentrate: roughage ratio as an amount of dry matter equal to 3% of their live body weight. The control group (G_1) was fed Concentrate Feed Mixture (CFM) and Rice Straw (RS) as the traditional feeding regimen in summer in Egypt. While, G_2 was fed CFM and untreated maize silage and G_3 was fed CFM and maize silage inoculated with LAB. Results indicated the digestibility of dry matter, organic matter, crude protein, crude fiber and nitrogen free extract regarding G_3 were higher ($p>0.05$) than those in G_1 and G_2, while, ether extract digestibility was unaffected by treatments. Milk yield and 4% Fat Corrected Milk (FCM) yield increased significantly with inoculated silage than the other treatments. Milk composition with G_3 was higher ($p<0.05$) for milk total solids, fat and lactose contents than those recorded for G_1 and G_2. Consequently, G_3 produced more yield of milk total solids, fat and protein ($p<0.05$) than the other groups. Some blood serum parameters were discussed as well. The inoculated maize silage group was better than the other two groups in respect of feed and economic efficiencies.

Key words: Lactic Acid Bacteria (LAB), inoculated maize silage, lactating buffaloes, digestibility, milk yield and composition, blood serum parameters

INTRODUCTION

Many factors related to maize silage preparing. Management can influence silage fermentation characteristics and its quality. Studies have demonstrated some of the chemical changes that occur in the corn plant as it is becoming mature and leads to less fermentable substrates being available for bacterial lactic acid fermentation and thus resulting low silage quality (McDonald *et al.*, 1991). They added Water-Soluble Carbohydrate (WSC) level is the most important substrate related to lactic acid fermentation process. Several studies have demonstrated the effect of bacterial inoculation into corn silage on silage fermentation characteristics (Higginbotham *et al.*, 1998; Cai *et al.*, 1999; Ranjit and Kung, 2000). Earlier criteria for the effective preservation of an ensiled crop included a high degree of lactic acid production and a pH below 4.2 after the fermentation phase (Bolsen *et al.*, 1996; Cleale *et al.*, 1990). Addition of bacterial inoculant containing homofermentative LAB improved the quality and aerobic stability of grass silage (Wrobel and Zastawny, 2004). With maize silage,

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Jatkauskas and Vrotniakiene (2004) revealed that the inoculant of LAB improved the fermentation quality, increased WSC and lactic acid contents and decreased amounts of acetic acid, butyric acid and ammonia-N. Its application demonstrated an improved intake and milk production with cows, compared to untreated silage. Vrotniakiene and Jatkauskas (2004) concluded that silage treated with the inoculant had a positive effect upon the quality of the fermentation process and positive influence on the bulls growth. Microbial additives improve silage quality, nutrient digestibilities and net energy for lactation (NEL), moreover, they reduce degradation of protein significantly (Pahlow and Honig, 1994; Ilakova *et al.*, 1998).

The objectives of this study were to investigate the effect of inoculated maize silage with LAB on the productive performance of lactating buffaloes.

MATERIALS AND METHODS

The present study was carried out in the experimental research station located in Shalakan, Faculty of Agriculture, Ain Shams University, Central laboratory for food and feed, Agriculture Research Center and Dairy Sciences Laboratory, National Research Center.

Experimental Animals

Nine lactating buffaloes at their 3rd lactation season and average weight 527±9 kg were chosen after two weeks of calving form the research station herd. They were divided into three similar groups of three animals each, according to their body weight and milk production, using complete randomized block design. Each group was assigned randomly to one of the three dietary treatments. The experimental animal groups were adapted on the experimental rations two weeks after the calving date and continued up to 105 days of lactation season.

Experimental Rations

The experimental rations were formulated to be 60:40 concentrate: Roughage ratio and offered to animals in order to be dry matter equal to 3% of their body weight. The Concentrate Feed Mixture (CFM) was composed of 30% yellow corn, 35% wheat bran, 25% undecorticated cotton seed meal, 3% rice bran, 3% molasses, 1% urea, 2% limestone and 1% mineral slats.

The control group (G_1) was fed CFM and rice straw as the traditional feeding regimen that applied in summer in Egypt. The (G_2) was fed (CFM) and untreated maize silage and (G_3) was fed (CFM) and maize silage inoculated with lactic acid bacteria. The type of inoculant used was called Pioneer 1132 which were composed of 100 billion CFU g^{-1} of crop specific *Lactobacillus planetarium* and *Enterococcus faecium*. The comparative feeding trail was extended for 90 days preceded by two weeks as adaptation period. The chemical composition of ration ingredients are presented in Table 1.

Management

Animals were individually fed and the daily concentrate feed mixture of each animal was divided into two similar parts. The first was offered before the morning milking (at 6.00 am), while the second one was offered before the evening milking (at 6.00 pm). Rice straw or maize silage was offered to the animals three times a day, at 8.00 am and at 2.00 and 8.00 pm The residues of feed were collected, determined and sampled for chemical analysis. Fresh water was available at all time.

Blocks of Vitamins and minerals mixture structured for dairy cattle were hanged in the front of each animal to be locked freely at all time. Buffaloes were machine milked twice daily at 6.00 am and 6.00 pm and milk yield was recorded for each buffalo.

Table 1: The chemical composition of the CFM, rice straw and uninoculated or inoculated maize silage (% DM basis)

Item	CFM	RS	Uninoculated maize silage	Inoculated maize silage
DM	91.29	92.85	32.88	33.52
OM	89.89	84.55	90.58	89.64
Ash	10.11	15.45	9.42	10.63
CP	14.14	3.50	9.53	9.35
EE	4.04	2.10	1.27	1.82
CF	15.33	33.90	24.62	23.84
NFE	56.38	45.05	55.16	54.36
NDF	37.90	79.13	77.44	68.84
ADF	22.10	52.32	37.53	33.62
ADL	4.50	5.50	4.48	1.90
Cellulose	17.60	46.82	33.05	31.72
Hemicellulose	15.80	26.81	39.91	35.22

CFM: Concentrate Feed Mixture, RS: Rice Straw, DM: Dry Matter, OM: Organic Matter, CP: Crude Protein, EE: Ether Extract, CF: Crude Fiber, NFE: Nitrogen Free Extract, NDF: Natural Detergent Fiber, ADF: Acid Detergent Fiber, ADL: Acid Detergent Lignin

Feedstuffs and Fecal Analysis

The chemical composition of different feedstuffs and residues were analyzed according to the AOAC (1995) methods for DM, CP, CF, EE and ash, while NFE and OM contents were calculated by difference. Fiber fraction (NDF, ADF and ADL) were determined according to Van Soest and Breston (1979). While, cellulose and hemicellulose were calculated by difference.

Digestibility Trial

Simultaneously three digestibility trail were carried out on all animals of each treatment of feeding trail and repeated each 30 days of the experimental period. Grab sample method was used and silica as internal marker was applied for determining the digestibility. Feces grab samples were collected handily at 8.00 am for three successive days from each animal. Solution of 10% H_2SO_4 were added to the representative samples then dried in oven at 70°C for 24 h. The dried feces samples from each animal were mixed and stored at -18°C for chemical analysis. The digestibility coefficient was calculated according to the following formula according to Gallup *et al*. (1945) and Forbes and Garrigus (1948).

$$\text{Digestibility} = 100 - \left[100 \times \frac{\%\ \text{Indicator in feed}}{\%\ \text{Indicator in feces}} \times \frac{\%\ \text{Nutrient in feces}}{\%\ \text{Nutrient in feed}}\right]$$

Sampling and Analysis of Milk

Milk sample were collected each two weeks during the experimental period. Composite samples were prepared by mixed 1% of each morning and evening milk yield. Milk samples were also, analyzed for fat, total solids (TS), total protein (TP) and ash (Ling, 1963). While, lactose was colorimetric determined according to Barrnett and Abd El-Tawab (1957). Fat Corrected Milk (FCM) was calculated according to Gaines (1928) equation as:

$$\text{FCM} = 0.4\ (\text{milk yield kg day}^{-1}) + 15\ (\text{milk fat yield kg day}^{-1})$$

Sampling and Analysis of Blood Serum

Blood samples were collected from the jugular vein of each animal at the last day of each period at 4 h post morning feeding. The collected blood samples were centrifuged at 4000 rpm/20 min to separate the serum. The obtained serum was stored at -18°C till it was analyzed. Glucose was determined as described by Trinder (1969) after collecting the sample. Serum total protein was determined as described by Gornall *et al*. (1949). Albumin was determined as described by

Doumas *et al.* (1971). Gobulin and albumin/globulin ratio were calculated. Serum total cholesterol determination was carried out as described by Allain *et al.* (1974). Urea was determined by the method of Chaney and Marbach (1962) and Triglycerides was determined by the method of Fossati and Prencipe (1982).

Statistical Analysis

The data were analyzed according to Statistical Analysis System (SAS) User's Guide (1996). Separation among means was carried out by using Duncan (1955) multiple tests used of least square of variance for repeated measures for data of digestibility trails and data of blood serum analysis, while complete randomized block design was used for data of milk yield and composition.

RESULTS AND DISCUSSION

Digestibility Coefficients

The inoculated group G_3 was higher ($p<0.05$) in DM and OM digestibility coefficients then G_2 and the later was higher ($p<0.05$) than G_1. Also, significant increases ($p<0.05$) were noted in CP, CF and NFE digestibility coefficients for the animals fed maize silage (G_2 and G_3) in comparison with control group (G_1). The inoculation had no significant effect on the digestibility of the previous nutrients (G_2 and G_3) (Table 2). There were significant increases ($p<0.05$) in Ether Extract (EE) digestibility by G_3 when compared to G_2 and control group. Several investigators were recorded an improvement in nutrients digestibility due to inclusion of silage ingredient fatting and dairy rations (El-Sayed *et al.*, 1997; Gaafar, 2001; Mostafa *et al.*, 2001). This improvements might be attributed that the reduction occurred in fiber content of hemicellulose during the ensiling process (Dewar *et al.*, 1963; Keady *et al.*, 1996) and a dramatic decrease in ADL content of inoculated silage Table 1. Therefore, it could be affect the digestibility to be better in G_3 than G_2. Supporting to the findings obtained here, Pahlow and Honig (1994) and Ilakova *et al.* (1998) found that the inoculation treatment of silage led to an improvement in silage quality and nutrients digestibility. Generally, Davies *et al.* (1998) concluded that the degree of improvement in nutrient digestibilities of inoculated silage to far extent depend on the availability degree of Water-Soluble Carbohydrates (WSC) in the biomass at silage fermentation phase. Raeth-Knight *et al.* (2007) found that no differences changes in apparent dry matter, crude protein, neutral detergent fiber, or starch digestibility among treatments when fed dairy cows on *Lactobacillus acidophillus* and *Propionibacteria freudenreichii.*

Table 2: Effect of different treatments on nutrients digestibility of lactating buffaloes during 105 day of lactation season

Items	G_1	G_2	G_3	±SE
Dry matter	59.04^c	63.14^b	72.84^a	1.55
Organic matter	59.90^c	63.60^b	74.52^a	0.86
Crude protein	65.06^b	71.12^a	72.33^a	1.97
Crude fiber	64.76^b	75.99^a	76.71^a	0.78
Either extract	64.61^b	64.34^b	70.10^a	1.78
Nitrogen free extract	65.04^b	74.46^a	76.83^a	2.34

$^{a, b}$ and ^{c} Means of treatments within same row with different superscript letter(s) differ ($p<0.05$)

Blood Serum Parameters

Glucose concentration was significantly higher ($p<0.05$) for group received corn silage with inoculants (G_3) than control group (G_1). Also, serum total protein concentration was higher with inoculated silage then other treatments (Table 3). These may be attributed to the higher nutrients digestibility which was recorded for the groups received the maize silage with or without inoculation.

Insignificant differences among treatments were observed in albumin, globulin, urea and total cholesterol. On the other hand, the control group was higher ($p<0.05$) in triglycerides values than the

Table 3: Effect of different treatments on blood serum parameters of lactating buffaloes during 105 days of lactation season

Item	G_1	G_2	G_3	±SE
Glucose (mg dL^{-1})	63.66^b	66.67^{ab}	72.17^a	3.24
Total protein (g dL^{-1})	6.22^b	6.39^b	6.98^a	0.14
Albumin (mg dL^{-1})	3.37	3.52	3.53	0.07
Globulin (mg dL^{-1})	2.80	2.87	3.44	0.21
Albumin : Globulin ratio	1.23^a	1.32^a	1.06^b	0.03
Urea (mg dL^{-1})	42.94	44.19	42.16	4.66
T. cholesterol (mg dL^{-1})	70.33	72.67	71.22	5.32
Triglycerides (mg dL^{-1})	18.87^a	15.10^b	14.62^b	1.01

$^{a, b}$ and ^{c} means of treatments within same row with different superscript differ ($p<0.05$)

Table 4: Effect of experimental rations on milk yield and composition of lactating buffaloes

Item	G_1	G_2	G_3	±SE
Milk yield (kg day^{-1})	6.90^b	7.35^b	7.58^a	0.833
4% FCM yield (kg day^{-1})	9.17^b	9.78^b	10.40^a	1.078
Milk constituents yield (kg day^{-1})				
TS	1.076^b	1.131^b	1.203^a	0.13
Fat	0.427^b	0.456^b	0.491^a	0.05
TP	0.293^b	0.315^b	0.322^a	0.04
Milk composition (%)				
TS	15.60^b	15.39^c	15.87^a	0.057
Fat	6.19^b	6.21^b	6.48^a	0.093
SNF	9.41	9.17	9.39	0.108
TP	4.24	4.28	4.25	0.012
Lactose	4.04^b	4.024^a	4.38^a	0.058
Ash	0.83^c	0.90^a	0.85^b	0.006

$^{a, b}$ and ^{c} means of treatments within the same row with different superscript are differ significantly ($p<0.05$). Ts: Total Solids, TP: Total Protein, SNF: Solids Not Fat

other groups. Albumin to globulin ratio ranged between 1.06 and 1.32 and this range are better agreed with that recorded by Khinizy *et al.* (1997) who fed buffaloe calves on maize silage ration. Moreover, Salem *et al.* (1989) reported that dietary protein and energy levels are the most effective factors in blood picture. Consistent with this conclusion, Mostafa *et al.* (2001) revealed that plasma total protein concentration was not affected significantly when grown bulls given isonitrogenous and isoenergetic rations irrespective the differences in ingredients that formulate a certain rations.

Milk Yield and its Composition

Actual milk and 4% FCM yields were significantly higher ($p<0.05$) for buffaloes fed inoculated silage ration (G_3) than those of control (G_1) or uninoculated silage (G_2). Also, significantly increases in actual milk and insignificantly increases in 4% FCM yields were noted with G_2 in relation to G_1 Table 4. The higher milk yield with the inoculated silage ration might be attributed to the positive effect of inoculation on the digestibility of organic matter and its nutrients Table 2. These results also probably attributed to the higher of glucose and protein concentration in the blood serum of G_3 Table 3. It led to an increase in milk lactose synthesis and consequently milk production being increase. In this respect, Yan *et al.* (1998) found that inoculation significantly increased the digestibility of organic matter, nitrogen, energy, NDF and ADF in the silage. Therefore, these results were inevitability increased the ruminant production.

Results of milk yield obtained in the present study are in agreement with those obtained by Pahlow and Honig (1994) and Ilakova *et al.* (1998) who stated that inoculants improved silage quality, nutrients digestibilities and net energy for lactation (NEL). Similar results were obtained by Jatkauskas and Vrotniakiene (2004) who demonstrated that inoculation improved DMI and milk production with cows compared to the uninoculated silage. On the other hand, Kent *et al.* (1988) and Kung *et al.* (1992) found no significant effect due to silage inoculants addition. Raeth-Knight *et al.* (2007) reported no differences effect in average dry matter intake or 4% fat-corrected milk when dairy cows fed *Lactobacillus acidophillus* and *Propionibacteria freudenreichii*.

Table 5: Feed efficiency and economic efficiency of the experimental rations

Item	G_1	G_2	G_3
Average body weight (kg)	542	518	521
Dry matter intake (kg day^{-1}):			
CFM	8.9	7.1	7.1
Rice straw	4.9	-	-
Uninoculated silage	-	5.1	-
Inoculated silage	-	-	5.0
Total DM intake (kg day^{-1})	13.8	12.2	12.1
Cost of FCM produced (LE)	11.96	12.81	13.11
Average of FCM (kg day^{-1})	9.17	9.78	10.40
Economic efficiency[1]	1.30	1.31	1.26
Feed efficiency[2]	0.66	0.80	0.86

[1]: Cost of kg FCM produced (LE), [2]: FCM/DMI

As an increase of milk yield, daily yields of milk TS, fat and TP were higher ($p<0.05$) with G_3 than those of other groups (Table 4).

Animals fed inoculated maize silage had higher ($p<0.05$) contents of milk total solids, fat and lactose compared to control group. Also, the TS content was declined ($p<0.05$), fat did not affected and lactose increased ($p<0.05$), with G_2 comparing with control (G_1). No significant differences were reported among treatments with respect to SNF and TP contents. On the other hand, G_2 was higher ($p<0.05$) in ash content than those of both G_1 and G_3. Results obtained here are in harmony with those found by Holden *et al.* (1995) and Zaki *et al.* (2001) who stated that no significant differences in milk constituents due to inclusion different proportions of corn silage in the dairy rations in comparison of the traditional ration that formulated from straw and concentrate mixture. Earlier similar results were obtained by Kent *et al.* (1988) and Kung *et al.* (1992). Raeth-Knight *et al.* (2007) reported that percentage or yield of milk components also did not differed when dairy cows fed *Lactobacillus acidophillus* and *Propionibacteria freudenreichii*.

Feed and Economic Efficiencies

Table 5 shows the average amount of daily dry matter feed intake (DMI) in the different groups during the experiment, feed efficiency expressed as kg 4% FCM kg^{-1} DMI produced and economic efficiency expressed as cost of one kg 4% FCM produced. It appears that the feed efficiency of G_3 is better than G_2 followed by G_1. It seamed that introducing the corn silage to the animals as an excellent roughage source decreased the amount of concentrates consumed by about 20.0% (from 8.9-7.1 kg day^{-1}) and raised the feed efficiency up to about 30.3% (from 0.66-0.86. It led to an increase in milk production up to about 9.85% in G_3 and 6.5% in G_2 than control. So the economical efficiency was markedly increased with the inoculated silage ration compared with other treatments. Economic efficiency was expressed as cost of feed required to produce one kg fat corrected milk. Assumption price (LE) of one ton of CFM, RS, uninoculated and inoculated maize silage were 1200, 60, 230 and 250, respectively. Brito and Broderick (2006) found that there were linear increases in apparent N efficiency and decreases in N excreted in urine and feces when corn silage replaced alfalfa silage of lactating dairy cows ration. While, Raeth-Knight *et al.* (2007) reported that feed efficiency averaged 1.52 kg of 4% fat-corrected milk/kg of DM intake and did not differ when dairy cows fed *Lactobacillus acidophillus* and *Propionibacteria freudenreichii*.

CONCLUSION

Using the inoculated whole maize silage as a good roughage source in the ration of lactating buffaloes improved their productive performance and economic efficiency of milk production.

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